DISEASE GERMS.

Another Illustration of the Fact that Bacteria Cause Disease.

By T. J. Burrill, Ph. D. Champaign, Ill.

Notwithstanding the numerous demonstrations of the active agency of micro-organisms in the production of disease, especially in animals, doubts are still expressed upon the subject in private interviews and in public prints. If these skeptical or combative utterances did not frequently come from those who are considered, however worthily, to a greater or less extent, authorities upon such matters, further contributions to the exposition of the truthfulness of the so-called germ theory of disease might not be considered necessary or useful. But there are to-day honest skeptics upon the subject who really wish for reliable information. Whether or not they have made reasonable use of the published account of experiments, whether or not they have embraced possible opportunities to experiment and investigate for themselves, need not now concern us. One who is convinced of a truth ought not to stand upon his dignity about publishing and republishing his reasons for the faith that is in him. There are other skeptics in the matter who apparently will not believe evidence on account of some previous occupation of the mind with other notions, the mental capacity perhaps not being sufficient for two ideas at the same time and the mental inertia (if one may so speak) too great for change when once started. As these latter intellects are not to be looked for within the membership of our organization, nothing further need be said here

to meet such opposition. To the former, if present, this communication may possess something of interest.

We certainly have some advantages in investigations of this kind in dealing with vegetables instead of animal bodies, on account of the comparative simplicity of structure and physiological function in the former. In plants each cell is, in an important sense, an independent physiological unit. One can cut a section from a living plant for microscopical examination and keep it under his eye for hours, while it retains its normal, vital condition and activities. There is no nervous system to complicate the problem and perhaps lead one to false conclusions as to the actual cause of the phenomena presented. In plants there are no so-called constitutional maladies, like ague and fevers. Disease is always local, at least in origin. If other parts are ever involved in consequence, it is only in a mechanical or physical way, as when by the want of proper root action the stem and leaves may suffer from the want of water, and not at all through sympathetic communication. Then again the structure of vegetable tissues is such that the elements can be much more readily examined by themselves. The cells are larger and more distinct as well as more permanent; the difference between wall and contents is greater, and the entire cell-structure is easier identified and examined.

To be sure, comparatively little has been done in the investigation of plant diseases due to bacteria; but this is sufficiently explained by the relatively few workers upon this department of plant pathology. While the earliest known disease of this kind upon plants was first announced in our country and to this society, there have been recorded in America, aside from the writings of the author, the results of but one series of careful investigations upon the relations of bacteria to vegetable disease, and this upon the same subject as that first presented, viz: the so-called fire blight of pear and other pomaceous trees.

This is by no means for want of opportunity upon the

material side. There certainly are enough plant diseases of the nature in question to furnish abundant chance for investigation. The failure is wholly upon the part of the investigators. Man's body is animal, not vegetable in make up. This in itself is sufficient to give extra stimulus to the studies upon the former kind of structures. There are hundreds and thousands of active workers whose professional business it is to deal with diseases of animals and man, and hundreds of special schools where instruction is directly imparted to students upon their diseases; but the professional vegetable pathologists are yet to be evolved, to say nothing of special schools for their training. Yet the time cannot be far distant when critical investigations will be made upon vegetable structures for the very purpose of helping towards the solution of the problems presented in animal pathology. This has been recognized by Sir James Paget whose paper upon the subject is interesting reading. As a contribution to the general doctrine of "disease germs" as well as for the intrinsic importance of the new results herewith presented, this paper is respectfully submitted.

During several years complaints have been made by those who grow crops of broom-corn and sorghum (*Sorghum vulgare*) of an injury especially evident upon young plants, but also upon those of any age, whereby great losses have occurred. Sometimes the unwelcome appearances of disease are confined to definite but usually irregular areas, and often within these areas the entire crop is destroyed. In other cases the diseased plants, in greater or less numbers and in various conditions of injury, are distributed throughout the field, smaller in size than the healthy ones, if any, and of a general sickly appearance. The lower leaves of the affected plants gradually die, but are first spotted and splashed with crimson-red, in all sizes and shapes. This conspicuous coloring is more particularly observed upon the upper portions of the leaf-sheaths which invest the stem, and, to a less extent, along the mid-veins of the leaves.

The stems themselves are not commonly damaged locally in a serious manner. If the stalks live to develop the brush or seed panicle, the peduncles or wiry stems of the latter are often badly scarred with irregular reddish patches. These may often be seen in manufactured brooms.

Upon examining the roots many of them are found dead. The affected plants are easily pulled up, often yielding to very slight force, while their healthy neighbors resist a vigorous pull. The oldest roots die first, and, as others are gradually emitted from the lower part of the stem in successive circles in the well-known order with these plants, they become successively diseased and die, so that only the youngest or those emerging highest upon the stem are still alive. There are no abrasions of the surface; as far as can be made out with a hand magnifier the tissues both of the leaves and of the roots remain intact until dead and dry, when the affected parts soon crumble away. Of the roots it seems to be only the exterior layer of tissue or cortex in which the disease is resident.

The injurious effects, now described, have been attributed to insects, to parasitic fungi, to unfavorable conditions of soil and of climate, and to constitutional weakness of the plants themselves. The crops are not comparatively important ones in this country at large and are usually locally cultivated, so that relatively little attention has been given to them by scientific investigators. But an account of careful studies upon the diseased condition of the plants and upon the insects infesting the fields is given in the Thirteenth Report of the State Entomologist of Illinois (1883) by Professor S. A. Forbes. These studies were avowedly unsatisfactory, but finding great numbers of plant lice, of four distinct species, in the fields, it was thought these or some of them probably caused the mischief. If however the injury could be assigned to the lice at all, Professor Forbes concluded that the main damage must have been done before the time of his examination and that the depredating

insects had largely disappeared, for their distribution at the time did not correspond with the evidence of damage done. He thought the trouble might be due to fungi, and specimens were sent me for examination, upon which a negative report was made.

I am not aware that the injury in question has elsewhere been attributed to specified insects.

In the *Prairie Farmer* for August, 1884 (Vol. LVI., p. 532), I gave a description of a fungus, supposed to be an unnamed species of Chætostroma, found in abundance upon affected leaves of broom-corn. This seemed at the time to cause at least some of the damage noted. It has been found several times since and probably does injure the crop, but cannot be connected in any way with the main disease with which we are now concerned. This completes, so far as I am informed, the accounts that have been published having reference to the particular and conspicous injury at present under discussion. If the references really are complete, we can understand that very little has been known upon a disease which has certainly existed at least six years in our country, and which was probably imported from abroad with the affected plants many years ago.

In July, 1886, I collected for microscopical examination some of the diseased plants and, upon using higher magnification than formerly, found numerous bacteria within the cells of the affected parts. An assistant in the Illinois State Laboratory of Natural History, Mr. Charles Woodworth, was then asked to make special experiments and observations. The results were speedily convincing that a specific micro-organism was in some way connected with the disease, and apparently as an active agent in its production. But for some reason, still unknown, after August 1st, 1886, the culture experiments undertaken were not successful, and the matter was dropped for the time, on account of other work. A pure culture of a Bacillus had, however, been made from

the affected plants, and the disease was experimentally produced by the use of this artificial culture. Sterilized water was used in exactly the same manner as a check and always without result.

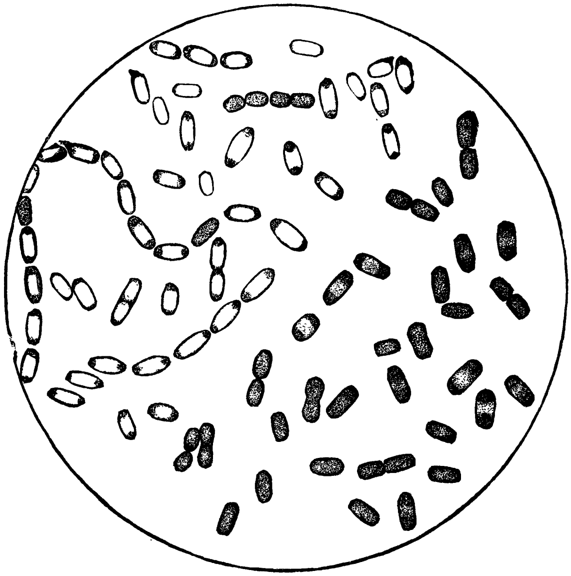
Wishing to ascertain the conditions of things in the winter, some broom-corn seed was planted in February, 1887, in the green-house, and April 6th, 1887, Mr. M. B. Waite, a student under my advice, commenced inoculation experiments upon the young plants with material taken from an old diseased stalk obtained at the time from the field. This old stalk contained, in great numbers, living microbes, though it was frozen hard when collected. There were also many spores recognizable by their peculiar refractive properties and uniform shape. The cultures from this old material were not always pure; but the prevailing organism was a Bacillus of apparently the same microscopical and culture characteristics as that found the year before. This organism was easily obtained in a state of purity by plate cultures and also was found to be pure in several direct transfers from the old stock, care being taken to effect this result.

The inoculation experiments upon the young plants were at once successful, both from macerations of the old diseased stock and from pure cultures of the Bacillus mentioned. Checks were made upon the same or similar plants by the use of sterilized water and of stertilized beef-broth like that in which the cultures were made. Studies were prosecuted until the first of June, when they were again interrupted, to be resumed a month later, by myself, aided by Mr. Waite, who had now become an assistant in the Illinois State Laboratory.

Without pausing now for the records of single experiments, a general summary of results is presented.

In the described disease of broom corn and sorghum a specific Bacillus is constantly found in the affected tissues, both of the roots and of the aerial parts of the plants. Pure cultures of this Bacillus may be made in beef broth, and in

infusions of potatoes and maize kernels, as well as upon nutrient gelatine and agar agar. The best growth takes place at a temperature of about 36° to 37° C., but development proceeds more slowly as the temperature is reduced below 25° C. Still lower temperatures were not tested. In potato infusion in a test tube, inoculated with a minute amount of a previous culture, or directly from the diseased tissues, and and placed in incubator at 36° C., the limpid fluid becomes sensibly turbid in twelve hours, and in twenty-four conspicuously so. In the latter case spores may be sparingly found. These are produced in a characteristic and uniform manner, one in the middle of each cylindrical segment of a chain, or in the individual microbe if separated. There is some undetermined condition under which the chains of cohering organisms are formed, for in some cases this is the prevailing form, while in others, apparently similar, the individuals are separated. In the most active stage of growth—say at twelve hours under the above conditions, the organisms are almost uniformly in pairs. In the preparation for spore-formation, changes take place in the protoplasmic contents of the cells indicated by the action of staining agents. During active growth methyl violet stains uniformerly and deeply the whole body. When spore formation begins, the central area of each cell is noticeably paler. At first this lighter colored portion looks like a pale, indistinct, equatorial band or zone without distinct limitations. Gradually the differentiation becomes more pronounced, until one sees a cylindrical cell with a dark spot in each end, and a comparatively large, white central area. By very careful examination, however, an exceedingly delicate film, seen in optical section as two fine border lines, can be made out as a lining of the cell wall throughout the white area. Sometimes the dark end spots appear like circular dots, but usually conform to the external shape of the cell



and are concave on their inner sides, or on those looking towards each other. These spots grow gradually smaller but do not wholly disappear, at least in many cases, until the cell-wall dissolves and leaves an oblong free spore entirely colorless except perhaps at the ends, where the violet stain still leaves its mark. When still older this agent does not color the spore. Aniline red with carbolic acid does stain them. The Bacillus averages $.7\mu$ in transverse diameter, but varies from about $.5\mu$ to 1μ. The joints are short, but run from 1 to 3μ in length, 1.5μ being more common. When as pairs newly divided the segments are oval but usually the shape is short - cylindrical. As the spores form, the sides become convex, so that the outline is elliptical, the ends however, remaining obtuse, or semi-circular. During the period of active growth, the organisms have flagellate motions, but these are never very rapid compared with many others, neither does the power seem to be long retained.

On plate cultures the characteristic growth is white or

pearl-like with peculiarly lobed and fimbriated margins. Gelatine is not liquified. In liquids, in the incubator as described, a pellicle forms upon the surface within twenty-four hours, but afterward becomes thicker. It is white or nearly so, usually polished or glazed above, with characteristic granules and pits. The growth extends upward on the sides of the test-tube about three millimeters. After a time the pellicle becomes brittle, easily breaks up and gradually settles to the bottom as a floculent precipitate.

When some of the culture fluid filled with the microbes is smeared upon the upper or under surface of healthy leaves of broom-corn or sorghum, after forty-eight hours minute red specks can be seen by the unaided eye. These specks are usually thickly dotted over the entire area to which the application was made; but, sometimes when the fluid settles more upon a given portion of the leaf, the spots are conspicuously more abundant there. By the aid of suitable magnification, it can be readily determined that the minute red specks owe their location to the stomates or breathing pores of the leaf. If a leaf is previously marked off by ink-lines into equal areas, say two inches wide, and, upon alternate blocks is painted in one case a fluid containing the Bacillus described, and upon the other a sterile fluid of the similar kind, the results are very striking and convincing. We find the former set speckled throughout with the red dots. At a later time, say four days, the peculiar checked appearance of the leaf corresponding to the treatment named, is conspicuously manifest, though not all of the tiny red dots subsequently enlarge. The infected surface becomes irregularly blotched. If now a portion of such a diseased leaf is slightly flamed, then cut with a flamed knife so as to divide the epidermis, and start a crack in the tissues when the leaf is bent, a freshly *broken* exposure of the diseased parts can be secured. A glass pipette just from the flame can now be thrust into the newly exposed reddish tissues and a culture started with pretty strong pre-

sumptions that whatever growth results, comes from the infected leaf. In this manner, time after time, a pure culture of the specific Bacillus has been secured and from these cultures the disease has been again started. Moreover the Bacillus itself has been found numerously enough in the affected tissues both when the disease occured spontaneously, that is naturally, and when artificially started as described.

But I have not yet learned to exhibit satisfactorily bacteria *in situ* with vegetable tissues. No stain or method of discoloring has been found servicable in these cases. Then too, the sections must be so thin that the cells themselves are usually cut and the contents escape. If infiltrated the mass interferes, or if the latter is dissolved the organisms are likely to be washed away.

If sections are made of a newly infected leaf, it is easy again to demonstrate that the disease starts at the stomates. The guard cells themselves may or may not be changed, but the cells immediately beneath the aërial cavity show the initial influence of the disease. From the stomates the injuries spread downward and sidewise. In thus spreading originally distinct spots coalesce and form continuous blotches as described.

The cell-walls are in no wise altered so far as can be made out by microscopical examination, except that they are stained throughout with red instead of their normal clear white. The first change observed in the cell-contents is a shrinking of the protoplasm, as when treated with alcohol. It separates from the wall of the cell and appears rigid instead of the usual plastic consistence in health. The chlorophyll granules, if present, lose their green color and break up into granules of much smaller size. The mass continues to shrink and becomes tinted with red. From this time on, the change does not appear to be always the same. Sometimes the shrunken mass seems tough and remains like a lump in the middle of the cell. In other cases it breaks up into granular débris, immersed in water.

If starch grains existed, they share the fate of the other material and are decomposed. At length the substance passes into what seems to be an emulsion of oily matter in water. The spherical particles are dark red and usually exhibit Brownian or molecular motion. In certain cells minute starch grains, of uniform size and shape, like little double-convex lenses, occur in great numbers and oscillate rapidly in the watery cell-fluids. These may be easily mistaken for microbes, but iodine stains them blue. They have been observed only near the borders of the diseased areas, within the parts which have turned red. Finally little or nothing remains within the inclosure of the cells; the surfaces of the wall, however, usually show what seems to be a granular or dirty deposit clinging to them. The cellulose of the cell-walls is stained beyond the area penetrated by the the microbes. The liquid itself in the diseased cells is red, colored thus by some soluble substance, perhaps a compound of tannic acid.

No attempts have been made to ascertain the chemical changes that take place either in the cultures or in the plants; but it is evident from what has been said that the injuries are chemical rather than mechanical. As before indicated the effect is, at first at least, purely local. It seems, however, quite probable that cells adjoining the invaded ones sometimes suffer from the absorption of the liquid only of the diseased parts. It indeed may be true that the protoplasm which shrinks into a lump and remains unchanged otherwise in the cell, is killed by the poisoned liquid and not by the direct action of the microbes. The latter have not been observed in such cells. The red coloring matter is not directly elaborated by the organisms, but results from the chemical decompositions of the cell-contents. The bacteria themselves are white and do neither absorb nor excrete the red stain. No culture fluid tried is changed in color by them.

The question is sure to be asked, "What are you going

to do about it?" The so-called practical man is very apt to say, "I care nothing about the cause. Tell me the cure." It is not always easy to say how an enemy can be safely met when he is seen; but it is true that a known foe is himself more exposed than one who fights under cover. The knowledge of the cause may lead to the cure.

If we concisely review the facts, remedial practices may be suggested. The destructive organisms infest any or all parts of the plants and live over winter, either as spores or otherwise, in the old material. In the case of broom-corn there is a large mass of this old substance left upon the ground, and it has been observed that since the improvement of plows permitting this refuse to be turned under, the disease has been much more destructive than when it was necessary to clear the ground by burning the old stalks. Undoubtedly one thing to do now, is to return to the former practice, and carefully burn the stubble. But this does not dispose of the old roots and underground parts of the stems which are infested with the parasites. Rotation of crops is better than trusting to burning, and field practice has given excellent demonstration of the utility of this system of management. Crops are sometimes injured on land not previously planted with sorghum or broom-corn; but the injury is invariably greater, so far as direct observation has been made, when successive crops of these plants are grown on the same land. The same microbe does not appear to affect maize, wheat or oats, though it is probable that it does live and prey on some other members of the great grass family. The most serious damage is done to the roots and no doubt these are far more liable to be infected from organisms already in the soil, than from such as might be washed down into fresh ground by rains. If the soil, on the other hand, contains great numbers of the living microbes, many of these will get into the air by the evaporation of water from the infested earth. This fact has been disputed, but experiments have repeatedly shown that bacteria may

be carried over in the practice of distilling water as well as disseminated in natural evaporation. Whether they ride on tiny droplets or are simply moved by the aerial currents produced, we need not stop to inquire. Their own power of movement is of course useless for such dissemination as we now consider.

It is quite possible that special fertilizers may be of service in checking the ravages of this disease, but nothing upon that subject is known. There seems to be no difference in regard to the general fertility of the soil. Indeed, the more luxuriant the growth, the more conspicuous the appearance of the disease upon artificial infection. Moist weather also seems favorable to the spread and abundant development of the malady. Rains appear to be efficient agents in the natural infections. During the unusual dry weather of the present season, the crops suffered little comparatively in the particular manner in question. Nothing has been noticed as to the difference, if any, in the different varieties in regard to the liability of infection Very probably there is some variation in this respect.

According to the tenth census of the United States there were produced in 1879, 12,792 lbs. of sugar and 28,444,207 gallons of molasses from sorghum and 29,480,160 lbs. of broom-corn. Counting the sugar at five cents per pound, the molasses thirty-five cents per gallon and the broom-corn at one hundred dollars per ton, and estimating the loss from this disease as five per cent. of the entire sum, which is certainly far within the actual amount, we have $571,506.00 as an annual tribute laid upon these comparatively unimportant crops in our country by the microscopic invaders, belonging to a single company of the mighty host which we are just beginning to recognize as warriors and enemies. Is it not time that we were opening our eyes and bestirring ourselves for a determined engagement? Victory ought to be, and may be, ours.

Having now presented the chief results of these investi-

tions, permit me to recall attention to the fact that this plant disease is started by simply placing the living microbes on the healthy leaves. There is no wound of any kind as a prerequisite. There is no preliminary letting down of constitutional vigor. The action of the bacteria is direct and indisputable. They, and they alone, must be considered the agents of the injury. It is by no means asserted that this is the first time such proofs have been offered, or that the work in this case has been exceptionally well done from the standpoint of general bacteriology; but, upon the principle of line upon line, the results must help to strengthen testimony already strong.

It only remains to introduce the new member of the family of known microbes as *Bacillus sorghi*, n. sp.